Central Valley Chinook Genetics Project Update

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The Central Valley Genetics Project began in May 1994 when DWR first contracted with UC Davis, Bodega Marine Lab (BML) to investigate the potential to distinguish between the chinook runs in the Central Valley using genetic characterization techniques. Dr. Dennis Hedgecock is the principal investigator for the study titled "Mixed Stock Analysis of Central Valley Chinook Salmon Using Short Tandem Repeat Nuclear DNA Polymorphisms." The study's goal is to find nuclear DNA markers sufficiently different to distinguish the four chinook runs, especially distinguishing winter chinook from the other three runs.

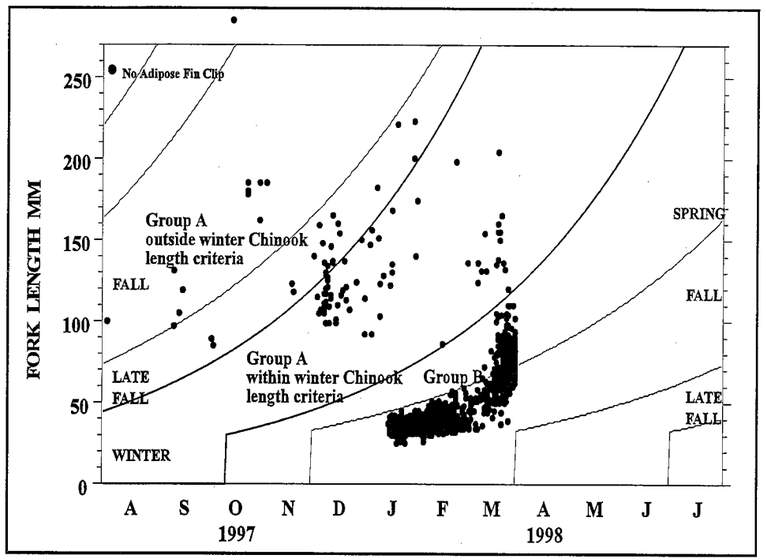

Figure 1
Observed chinook salvage at the SWP and CVP delta fish facilities 8/1/97 through 3/31/98.

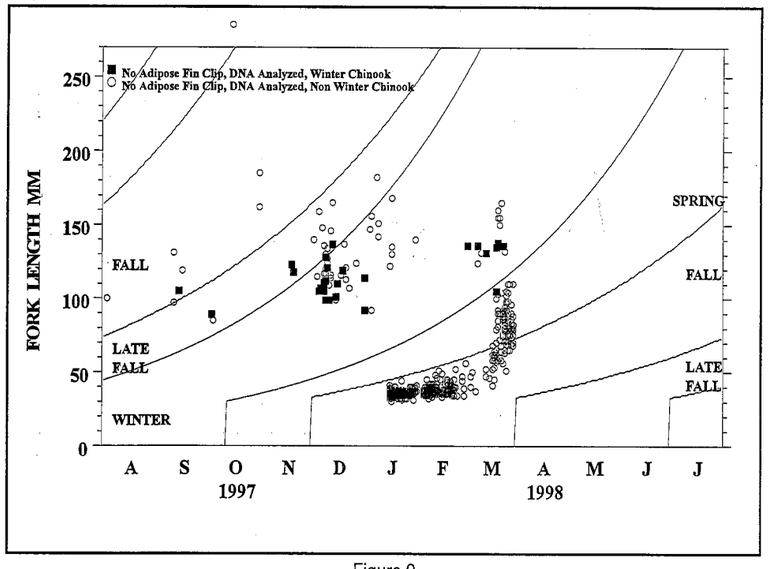

Figure 2
DNA analyzed chinook salvage at the SWP and delta fish facilities 8/1/97 through 3/31/98.

BML focused on microsatellite regions of the nuclear DNA because microsatellites have properties suited for distinguishing between closely related populations. Some of these properties are a rapid mutation rate, highly conserved mutations, and Mendelian inheritance. The first contract was for three years, during which BML isolated and evaluated microsatellites for distinguishing capabilities, developed winter, spring, fall and late-fall baselines from chinook of known origin, optimized a statistical procedure called Mixed Stock Analysis (MSA) to estimate the run composition of a chinook population composed of all four runs, and developed a mathematical likelihood technique to determine the run identity of individual chinook.

Early results were encouraging. BML isolated one marker highly distinct for winter-run chinook, *Ots*-2, and identified another marker originally from sockeye salmon, *One*-13, also distinguishing for winter-run chinook. BML isolated and evaluated several other markers that, when used in a MSA, provided sufficient power to distinguish with a high degree of confidence the proportion of winter-run chinook in a mixed population. Throughout this time, they were developing a mathematical likelihood technique to identify winter-run chinook on an individual basis.

Table 1. Results of Mixed Stock Analysis on SWP and CVP Chinook Salvage from August 1997 through March 1998 Using Microsatellite Allele Frequency Genetic Characteristics

	% Winter ChinookUsing Former Winter Chinook Baseline	% Winter Chinook Using New Winter Chinook Baseline
GROUP A (Chinook larger than minimum winter Chinook length criteria), n = 80		
Chinook within winter Chinook length criteria, n = 57		
Estimate	44.22%	40.64%
Standard error	0.0720	0.0699
Chinook outside winter Chinook length citeria, n = 23		
Estimate	26.37%	21.65%
Standard error	0.0959	0.0923
GROUP B (Chinook smaller than minimum winter Chinook length criteria), n = 592		
Estimate	n/a	0.17%
Standard Error	n/a	0.0017

The initial focus on winter-run chinook was due to an immediate management need to distinguish winter-run chinook salvaged at the SWP and CVP export facilities in order to estimate the impact of water exports on winter-run chinook. DWR and the IEP also have been interested in using genetic characterization to provide additional information important to help answer many chinook life history questions, including rearing and emigration of all four Central Valley runs.

In 1995, BML encountered a problem in the winter-run chinook baseline that delayed progress on the MSA and individual identification technique. During an investigation of broodstock for the Winter-Run Chinook Propagation Program, they found genetic evidence that spring-run chinook had been inadvertently mixed with winter-run chinook; the genetic term is admixture. Using the very preliminary individual identification technique, BML determined which individuals were more likely spring-run chinook. Upon further investigation, BML determined a few spring-run chinook were among the winter-run chinook baseline collected in most years. Since individual identification is difficult among closely related runs, BML used a variety of statistical methods to resolve the run identities of the winter-run chinook baseline (Calavetta et al. 1998).

BML recently completed its investigation, and is confident the revised winter-run chinook baseline is composed of only winter-run chinook. The lab proceeded with a final MSA for the proportion of winter-run chinook in a mixed population and a preliminary individual identification for winter-run chinook. They will perform similar admixture investigations on the baselines for the other three runs.

BML performed MSAs on SWP and CVP salvage from August 1997 through March 1998 using the new winter-run chinook baseline. The SWP and CVP salvage was divided into two groups for the MSAs. Group A was composed of a small number of chinook larger than the minimum winter-run chinook length criteria, and Group B was composed of a very large number of chinook smaller than the minimum winter-run chinook length criteria. IEP also subdivided Group A into those within the winter-run chinook length range and those outside the winter-run chinook length criteria (Figure 1).

The IEP expected to find most of the winter-run chinook to be in Group A and very few in Group B. BML analyzed all of the samples in Group A, but due to the very larger number of samples in Group B, we randomly selected 25% for analysis. As BML expected, the new MSA results were very similar to the preliminary results (Table 1) (Calavetta et al. 1998). The results are in terms of winter-run chinook versus nonwinter-run chinook while the baselines for the other three runs are investigated for admixture.

They also performed a very preliminary individual identification analysis on the same 1997/98 SWP and CVP salvage samples. Again, the results are in terms of

Table 2. Results of Mixed Stock Analysis and Individual Identification Analysis on SWP and CVP Chinook Salvage from August 1997 through March 1998 uSing Microsatellite Allele Frequency Genetic Characterization

	Mixed Stock Analysis	Individual Identification Analysis
GROUP A (Chinook larger than minimum winter Chinook length criteria), n = 80		
Chinook within winter Chinook length criteria, n = 57		
Estimate	40.64%	38.60% (22)
Standard error	0.0699	n/a
Chinook outside winter Chinook length criteria, n = 23		
Estimate	21.65%	33.75% (27)
Standard error	0.0923	n/a
GROUP B (Chinook smaller than minimum winter Chinook length criteria), n = 592		
Estimate	0.17%	0.17% (1)
Standard Error	0.0017	n/a

winter-run chinook versus nonwinter-run chinook. The individual identification analysis uses a likelihood technique (analogous to betting odds). The appropriate critical minimum value that should be used to identify a winter-run chinook will depend on the management issue.

For this preliminary analysis, the lab used a critical value of 1, which means an individual is identified as be a winter-run chinook if the likelihood that it is a winter-run chinook is greater than or equal to the likelihood that it is one of the other runs. The 1:1 odds were considered adequate for this preliminary analysis, realizing a statistical justification for the critical minimum value will be necessary to evaluate the management issue. The results of the individual analysis corroborated the results of the MSAs (Table 2).

The winter-run chinook, based on genetic characterization, are illustrated in Figure 2. As expected, almost all of the winter-run Chinook were in Group A, but one was in Group B, close to the line that delineates the two runs. The winter-run chinook were salvaged within the time expected, but most of them were salvaged earlier than expected based on our current understanding of winter-run chinook life history. This preliminary analysis represents only one season of data, and BML is proceeding with analysis on the previous years of salvage, delta monitoring, and upper Sacramento River data. The Genetics PWT will review and discuss the genetics results and their application to the SWP and CVP salvage and chinook monitoring programs.

This year, IEP planned to demonstrate the genetic analysis in real time using the SWP and CVP salvage samples. The first set of tissue samples was sent to BML in December 1997. At first there were some logistical and technical problems with both the genetic analysis and procedures for tissue collection, archiving, and distribution. Close coordination between staff at DFG Region 2, DWR ESO and BML resolved these problems with each successive set of samples throughout the next three months. BML reported genetic results for the set of 200 samples sent in April in four days, demonstrating genetic identification of chinook can be completed a in "rapid response" time frame when immediate management responses are necessary.

The next step is to apply the genetic analysis to the samples collected throughout the last three years. Tissue sampling at the delta pumping facilities and in chinook monitoring programs began in 1995. There are samples collected at the SWP from late spring 1995 through early spring 1996, and from fall 1996 through spring 1997, as well as samples collected in the Sacramento-San Joaquin Delta and in the upper Sacramento River from fall 1995 collected four times a year. Both the MSA and individual identification analysis will be applied to these sample collections. The Genetics PWT will discuss the genetics results and their application to the SWP and CVP salvage and Chinook monitoring programs.

An opportunity to test the accuracy of the genetic methodology occurred through a related genetic project, the Winter-Run Chinook Captive Broodstock and Propagation Program. In spring 1997, USFWS trapped fish in Battle Creek to relocate any winter-run chinook from the Propagation Program returning to Battle Creek to the mainstem Sacramento River to spawn. BML used the preliminary individual identification analysis to identify winter-run chinook, and among the Propagation Program winter-run chinook, to identify suspect hybrids of winter- and spring-run chinook. USFWS held adult chinook trapped in Battle Creek at the Coleman hatchery while BML performed the genetic analysis.

The Propagation Program and BML took this opportunity to test the accuracy of the individual identification technique under field conditions requiring very "rapid response." The samples, delivered to BML, were in unlabeled vials to prevent BML from knowing the run identity before the analysis. First, BML performed the individual identification analysis and reported to the USFWS whether or not the individual was a winter-run chinook. Subsequently, USFWS reported to BML whether the individual was adipose fin clipped and of hatchery origin.

Second, BML performed the individual parentage analysis and reported to USFWS whether the adipose fin clipped individual was from the Winter-Run Chinook Propagation Program and whether it was a suspected hybrid. By the end of the 1997 trapping, BML reported genetic results in three days, demonstrating again that genetic identification of chinook can be completed in a "rapid response" time frame.

BML identified 81 adipose fin clipped fish as winter-run chinook, and subsequently identified their propagation program family. USFWS sacrificed 13 of these and confirmed them as winter-run chinook, and relocated the remaining 68 to the mainstem Sacramento River to spawn. BML identified 16 adipose fin clipped fish as nonwinter-run chinook. USFWS sacrificed all of these and confirmed them as nonwinter-run chinook. BML identified one adipose fin clipped fish as fall-run Chinook. When USFWS sacrificed the fish, they confirmed it as one of the suspect winter/spring hybrids; these genetic characterization techniques tend to fail on hybrid populations.

In fall 1997, the Genetics PWT proposed a test of the ability of BML to reproduce the results of the genetic laboratory techniques and the computer algorithms used in the individual identification technique. We designed the test for the Winter-Run Chinook Propagation Program, and selected 95 random samples from the adults of known origin archived at DFG Region 2. The samples were in unlabeled vials, to prevent BML from knowing the run identities of the samples. The 95 samples were from the four chinook runs from stocks that occur in the upper Sacramento River. Twenty-nine were from the winter-run chinook baseline, and 22 each from the spring, fall and late-fall Chinook baselines.

The results are in terms of winter-run chinook versus nonwinter-run chinook. BML correctly identified each of the 67 samples they were able to amplify, however, they were unable to amplify and therefore analyze 28 samples. The simplest explanation for this was that the DNA in the samples had degraded. The Genetics PWT will investigate to try and determine the reason these samples did not amplify, whether that be field collection, short term storage, long term archive or laboratory procedures.

During the process of trying several DNA extraction methods on the difficult test samples, BML discovered an intriguing genetic phenomenon, known as a "paralogous" locus. The *Ots-2* locus, originally assumed to be unique, was found at another location on the DNA, presumably originating from tetrasomy. BML redesigned the amplification primers to exclude the "paralogous" locus, and proceeded with the original genetic analysis. They will publish this finding in the genetic literature (Banks et al. 1998.)

Two other genetic projects are related to the Central Valley Chinook Genetics Project: a Spring Chinook Genetics Project, and a Winter-Run Chinook Captive Broodstock and Propagation Program Genetics Project. Following are updates on these two projects.

The Spring Chinook Genetics Project objectives are (1) to focus on identifying genetic markers to distinguish spring-run chinook from the other Central Valley runs; (2) among the spring-run chinook stocks, develop and optimize biochemical/genetic laboratory techniques to maximize the efficiency of genetic characterization of chinook and other salmonid species; and (3) perform genetic characterization on experimental ocean fisheries.

Through the Spring Chinook Genetics Project, BML developed a technique to extract large amounts of long strand DNA from carcass tissue, and to use a nonradioactive enrichment screening method to detect microsatellites in chinook. Using these new techniques, they were able to increase their efficiency in creating libraries of microsatellites for spring-run chinook, steelhead, and coho. BML sequenced several microsatellites and is screening them for their capability to distinguish among stocks. To maximize the efficiency of microsatellite amplification, BML redesigned several primers, used to am-

plify specific microsatellite markers, to amplify more than one microsatellite marker in one reaction. This reduced the number of amplification reactions and sequencing gels from five to two. BML designed an additional primer for a DNA gene, developed by Dr. Phil Hedrick, found to have distinguishing capabilities in chinook (Banks 1998).

Also through the Spring Chinook Genetics Project, BML performed genetic characterization of chinook captured in two experimental ocean fisheries conducted in 1997. They focused on identification of Central Valley spring- and winter-run chinook in the experimental ocean fisheries composed of stocks from southern Oregon and California. BML performed a MSA on allozyme data acquired through NMFS and estimated greater than 95% of the catch was from the Central Valley. They compared the allozyme results with a MSA and individual identification analysis using the microsatellite markers developed at the lab. The preliminary results were less than 1% of the experimental ocean fisheries were winter-run chinook from the Central Valley (Banks 1998).

The Winter-Run Chinook Captive Broodstock and Propagation Program began a year before the Central Valley Chinook Genetics Project. The goal is to maintain the genetic integrity of winter Chinook both in the Captive Broodstock, the Propagation Program, and in the wild.

Through the Winter-Run Chinook Captive Broodstock and Propagation Program Genetics Project, USFWS and BML repeated the Battle Creek trapping and "rapid response" genetic identification (described above) in 1998. BML streamlined the procedure and was able to report genetic results one day after receiving tissue samples, demonstrating again, genetic identification of chinook can be completed rapidly. USFWS restarted the artificial propagation program in 1998, after a two-year suspension. This year, BML will perform the preliminary individual identification analysis to identify winter-run chinook to minimize the possibility of hybridizing winter-run chinook with another run. in preparation for the potential use of gametes from the Winter-Run Captive Broodstock in the Propagation Program, BML is determining the parentage of all captive chinook available for spawning in 1998 (Rashbrook 1998).

Literature Cited

Calavetta, M., C. Dean and D. Hedgecock. 1998. *Microsatellite DNA for* Management and Protection of Endangered Central Valley Chinook Salmon. Progress Report.

Banks, M., C. Greig, M. Barton and D. Hedgecock. 1998. *Molecular Genetic Identification of Chinook Salmon Runs Focusing on Spring Run Integrity.* Progress Report.

Rashbrook, V., H. Fitzgerald and D. Hedgecock. 1998. *Genetic Maintenance of Hatchery- and Natural-Origin Winter-Run Chinook Salmon.* Progress Report.

continued from page 4

5000 fish need processing of their code-wire-tags before the results of the fall run survival experiments are known.

Even with this doubled effort at Chipps Island, only 31 winter run sized chinook were captured during this period versus 46 winter-run sized chinook last year. Fall-run chinook catches, however, totaled 26,688 during this increased effort as compared to 2,575 captured last year during our regular sampling effort. Even taking the double effort into consideration, fall-run catches are much higher this year at Chipps Island. Delta smelt catch limited trawling in the final week of June, when one day of trawling was suspended as we reached our weekly limit.

Analysis of Existing Data on Shallow Water Fish Habitat

Mike Chotkowski

This study is designed to provide information about the use of shallow-water habitats by fish in the Sacramento-San Joaquin Estuary through analyses of existing data on fish collections made in shallow water. More than 10 such databases (collected by IEP agencies) exist, spanning periods of one to many years between 1959 and the present. The principal objectives of this study include (1) consolidating and formatting the available databases and constructing a descriptive database to serve as a key to records in the others; (2) constructing an inventory of fish species and life stages that use historically sampled shallow-water habitats, including timing of use; (3) summarizing shallow-water habitat types that have been sampled, and those that have not, for future use; (4) statistically analyzing fish databases, using relevant physical and other biological databases. Besides the summary database, an IEP technical report will be produced; peer-reviewed journal articles are also possible.

This study is being conducted by Mike Chotkowski (DFG), with work to date focused mainly on objectives (1) and (2). A steering committee has been formed to plan statistical analyses and will meet for the first time on 9 July 1998. Final products are due in 1999.

What's New on the Mitten Crab Front?

Tanya Veldhuizen, DWR, and Kathy Hieb, DFG

The Chinese mitten crab, *Eriocheir sinesis*, has rapidly increased its distribution in the San Francisco Estuary and watershed since it was first discovered in south San Francisco Bay in 1992. As of July 1998, the known distribution of the Chinese mitten crab extends north to Hunter's Creek (near Delevan National Wildlife Area) in the Sacramento River drainage and near Nicolaus in the Feather River, east to Roseville (Cirby Creek) and eastern San Joaquin County (Escalon-Bellota Weir on the Calaveras River and Littlejohns Creek near Farmington) and south to the San Luis National Wildlife Refuge near Gustine. We also have an unconfirmed report from the lower Stanislaus River. The mitten crab's distribution is also expanding in tributaries to San Pablo Bay, with sightings from all the major tributaries to Petaluma Creek and from a tributary to Sonoma Creek near Sonoma. It has been found throughout the Delta and South Bay tributaries.

Any crab found in fresh water is likely to be a mitten crab. The main identifying characteristic of the mitten crab is brown "hair" on the front claws (see figures below). Very small juveniles (<25 mm carapace width) rarely have "hairy" claws and may be confused with another non-native crab, the Harris mud crab (*Rithropanopeus harrisi*).

If you find a mitten crab beyond the current known range, please notify Kathy Hieb (khieb@delta.dfg.ca.gov) or Tanya Veldhuizen (tanyav@water.ca.gov) with the collection information (i.e., date, location, size, number, collection method, and contact person). You do not need to send us the crab.

Remember, it is illegal to import, transport, or possess live Chinese mitten crabs (Title 14 of the California Code of Regulations). Accidental release or escape will spread these crabs to uninfested waters. If you keep a mitten crab, it must be dead.